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13. ABSTRACT (Maximum 200 Words) The purpose of this research is to develop the infrastructure for comparative studies of prostate cancer among blacks who reside in contrasting environmental settings, West Africa, the Caribbean and the United States. This effort addresses six areas: case recruitment, case characterization, tissue collection and storage, integrated database development, targeted laboratory expertise and pilot research. <u>Key Research Accomplishments-Year 3:</u> 1) Established a research infrastructure that supports unified measurement of exposure and prostate cancer disease in Chicago, Illinois and Kingston, Jamaica; 2) Completed molecular in over 40% of subjects enrolled; 3) Created a computerized database linking demographical, clinical and pathological characteristics of each case to archived tissue specimens and results of nutritional and genetic measurements; 4) Completed statistical comparisons of i) demographical, clinical and pathological characteristics of cases from Chicago, Kingston and West Africa, ii) levels of antioxidants and fatty acids in serum and prostate tissue in cases diagnosed in Chicago and Jamaica, iii) and have performed association studies between variants of genes involved in androgen metabolism and clinical stage of prostate cancer within and across cases from Chicago, Jamaica and West Africa; 6) Our first manuscript was accepted to the journal <u>The Prostate</u> , and initial findings from our pilot studies have been presented at national meetings. The remainder of the no-cost extension will be used to complete and submit manuscripts and research grant proposals based on our work thus far.				
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I. INTRODUCTION

The purpose of this research is to develop the infrastructure for comparative studies of prostate cancer among blacks who reside in contrasting environmental settings, West Africa, the Caribbean and the United States. This ambitious three-year effort addresses six essential infrastructure areas: case recruitment, case characterization, tissue collection and storage, integrated database development, targeted laboratory expertise and pilot research. Per the previous two annual reports, our key accomplishments have been as follows:

1. Reliable recruitment and data collection strategies in Chicago and Kingston.
2. A centralized data repository in Chicago consisting of demographic and clinico-pathologic history and tissue (serum/plasma, leukocytes, erythrocytes and prostate tissue) for biochemical and molecular studies.
3. Meeting solicitation and recruitment targets in Chicago and Kingston.
4. Establishment of secure web-based technology to solve the problem of pathologists assigning histological grades of each case enrolled by consensus.
5. Positioned to conduct preliminary biologic and molecular comparison of cases in from Chicago and Kingston.
6. Two abstracts published and two manuscripts under review.

However, two of our most important problems have been:

1. Interruption of enrollment in Jamaica for 18 months (9/00-2/02) and in West Africa since September 2000 because of an appropriate administrative apparatus for the protection of human subjects at each site had not yet been verified by the PI and approved by HSRRB.
2. An unreliable co-investigator and internal politics at the University of Ibadan, Nigeria making it difficult to establish credible research operations in the region.

The necessary project assurances to resume subject enrollment and data collection in Kingston, Jamaica were eventually obtained, and in May 2001 we made the decision to transfer our West African operations from Ibadan, Nigeria to Accra, Ghana. Other changes proposed included extending recruitment to a busy private urology practice affiliated with Mercy Hospital on Chicago's Southside to enhance enrollment of African-Americans. These and other amendments to the original protocol were approved by Loyola's IRB toward the end of the project's second year (1/16/02). *A 12-month no-cost extension of the project was granted to 1) complete recruitment of West African cases; 2) perform additional genetic, nutritional and clinical pilot studies; 3) clean and analyze the data; 4) prepare and submit additional manuscripts.*

Adjustments to our research strategy in response to ever evolving challenges on the ground has enabled us to maintain the scientific validity of this study, demonstrate its usefulness as a model for comparing prostate cancer across populations from different environments as well as

preliminarily test some interesting hypotheses. Before describing this progress further using the required format, I will summarize the amendments made to our original Statement of Work (Table 1), specify the number of subjects enrolled under each protocol by site (Table 2) and present a timeline summary of work completed since last our report.

TABLE I. Statement of Work - Original and Amendments

	ORIGINAL	AMENDMENTS
Statement of Work	<p>Task 1. Provide reliable recruitment of incident cases in region.</p> <p>a) Create consortia of urologist and pathologists in each region: SW Nigeria (incl. Ibadan and Lagos), Jamaica and Chicago, IL.</p> <p>b) Develop incident case recruitment strategies appropriate for each research site, with the goal of <i>soliciting participation</i> of 75% of newly diagnosed cases per site (25-50/region) per year.</p> <p>Task 2. Characterize each case using a common protocol.</p> <p>a) Convene pathologists for a review of the Gleason grading system and group reading of representative slide of cases diagnosed in each region.</p> <p>b) Determine histologic grades ('Gleason sums') of cases subsequently enrolled by consensus via the Internet, using whole slide images created by Bacus Laboratory Inc. and posted on an access-restricted website.</p> <p>c) Identify and monitor adherence to a common set of tumor and lymph node staging procedures.</p> <p>d) Collect baseline demographic, clinical and pathologic data via medical records review and by patient interviews where needed.</p> <p>Task 3. Create a centralized repository for serum, plasma, leukocytes and prostate tissue for biochemical and molecular studies.</p> <p>a) Collect plasma, serum, and leukocytes on each case at the time of diagnosis, as well as fresh normal prostate tissue at the time of surgery from those undergoing radical prostatectomy</p> <p>b) Bank all specimens in Chicago (Department of Preventive Medicine, Loyola University) using an existing barcode driven specimen identification and storage system.</p>	<p>Task 1:</p> <p>a) Per reasons cited in the previous two annual progress reports, attempt to transfer research operations in West Africa from the University of Ibadan in Nigeria to Korle Bu Teaching Hospital of the University of Ghana in Accra, Ghana. Dr. Samuel Gepi-Attee, a urologist, will serve as the regional investigator for the study; a pathologist at Korle Bu will be named at a later date. Meanwhile, extend recruitment of eligible African-American men to Prairie Medical, a busy private urology practice on Chicago's predominantly African-American Southside affiliated with Mercy Hospital.</p> <p>b) Since initial recruitment in Chicago and Jamaica tended to exceed expectations, increase the total number of cases to be recruited in each region will be Increase from 60 to 120 per year.</p> <p>Task 2:</p> <p>b) Per reasons cited in the previous report, replace Dr. Eva Wojcik with an outside pathologist who will serve as the pathology consultant for the project. Along with his/her colleague in Jamaica and Ghana, this new pathologist will be responsible for making histopathologic determinations on cases enrolled in Chicago area [Loyola University Medical Center/Edward Hines VA and Mercy Hospital] and to participate in the protocol to monitor inter-observer agreement. Level of agreement between pathologists will be monitored using a 25% random sample from each site to be circulated to each pathologist. Determining Gleason sums on all cases by consensus between pathologists will no longer be attempted.</p> <p>Task 3:</p> <p>a) Collect height and weight at baseline, and follow-up data on symptoms, response to treatment, recurrence/progression, vital status and causes of death using a structured questionnaire.</p> <p>b) Process and store all patient specimens in a -70° C freezer Edward Hines, Jr. VA, in Building 1, Room C208.</p>

TABLE I. Statement of Work - - Original and Amendments (Continued)

ORIGINAL		AMENDED																																						
Statement of Work	<p>Task 4. Link case demographic, clinical and pathologic characteristics to corresponding tissue samples using a computerized database.</p> <p>a) Establish a single computerized registry of demographic, clinic and pathologic data for cases recruited in each region.</p> <p>b) Combine tissue and registry data into a single electronic record, linking case registry information to corresponding tissue samples using their unique barcode identification number.</p>	<p>Task 4.</p> <p>No changes requested.</p>																																						
	<p>Task 5. Pilot Studies: Conduct comparative studies of genes, nutrition and histopathologic markers of prognosis.</p> <p>a) Compare androgen receptor gene CAG repeat sequence lengths and the distribution of CYP3A4 receptor gene variants among 50 cases vs. 50 age-matched controls in each region, and how they relate to stage at presentation within and between groups.</p> <p>b) In 20 of these men undergoing radical prostatectomy in each region, measure prostatic levels of carotenoids, tocopherols, retinol and fatty acids. Compare mean levels, and explore how they relate to markers and whether they modify a relation between androgen and CYP3A4 receptor gene variants and markers of progression, raising the possibility of gene-nutrient interactions.</p>	<p>Task 5.</p> <p>Restructure Pilot Studies as Follows:</p> <table><tr><th>Study Purpose</th><th>Design</th><th>Endpt.</th><th>Feature or Risk Factor</th><th>Within Sites</th><th>Across Sites</th></tr><tr><td rowspan="2">I. Descriptive</td><td>Frequency Distribution</td><td></td><td>Clinico-pathology, Genes, Nutrition, Metabolism</td><td>Yes</td><td>Yes</td></tr><tr><td rowspan="3">II. Analytic</td><td>A. Disease Prognosis</td><td>Cross-sectional</td><td>Inter-mediate Endpoint</td><td>Genes, Nutrition, Metabolism</td><td>Yes</td></tr><tr><td>Retrospective cohort</td><td>Clinical outcome</td><td>Genes, Nutrition, Metabolism</td><td>Yes</td><td>Yes</td></tr><tr><td>Prospective cohort</td><td>Clinical outcome</td><td>Genes, Nutrition, Metabolism</td><td>Yes</td><td>Yes</td></tr><tr><td rowspan="2">B. Disease Etiology</td><td rowspan="2">Traditional case-control studies</td><td>Disease present</td><td>Genes, Nutrition, Metabolism</td><td>Yes</td><td>Yes</td></tr><tr><td>Inter-mediate Endpoint</td><td>Genes, Nutrition, Metabolism</td><td>TBD</td><td>TBD</td></tr></table>	Study Purpose	Design	Endpt.	Feature or Risk Factor	Within Sites	Across Sites	I. Descriptive	Frequency Distribution		Clinico-pathology, Genes, Nutrition, Metabolism	Yes	Yes	II. Analytic	A. Disease Prognosis	Cross-sectional	Inter-mediate Endpoint	Genes, Nutrition, Metabolism	Yes	Retrospective cohort	Clinical outcome	Genes, Nutrition, Metabolism	Yes	Yes	Prospective cohort	Clinical outcome	Genes, Nutrition, Metabolism	Yes	Yes	B. Disease Etiology	Traditional case-control studies	Disease present	Genes, Nutrition, Metabolism	Yes	Yes	Inter-mediate Endpoint	Genes, Nutrition, Metabolism	TBD	TBD
	Study Purpose	Design	Endpt.	Feature or Risk Factor	Within Sites	Across Sites																																		
	I. Descriptive	Frequency Distribution		Clinico-pathology, Genes, Nutrition, Metabolism	Yes	Yes																																		
		II. Analytic	A. Disease Prognosis	Cross-sectional	Inter-mediate Endpoint	Genes, Nutrition, Metabolism	Yes																																	
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B. Disease Etiology	Traditional case-control studies	Disease present	Genes, Nutrition, Metabolism	Yes	Yes																																			
		Inter-mediate Endpoint	Genes, Nutrition, Metabolism	TBD	TBD																																			

TABLE II. Protocols and Subject Recruitment:

	<u>ORIGINAL PROTOCOL</u>		<u>AMENDED PROTOCOL</u>			
Sites w/ Local IRB Approval	*Loyola University of Chicago (LUMC) (Approval date: 10/99)		*Loyola University of Chicago (LUMC) (Approval date: 1/02)			
	*Mercy Hospital (Approval date: 10/01)		*Mercy Hospital (Approval date: 10/02)			
	*The University of the West Indies, Jamaica (Approval date: ~2/2001)		*Korle Bu Hospital, University of Ghana, Accra, Ghana (Approval date: 10/02)			
	*University of Ibadan, Nigeria (Approval date: Per report of regional PI; actual date not known.)		*The University of the West Indies, Jamaica (Enrollment ended as scheduled but before the amended protocol was approved; hence, all of the participants in Jamaica were enrolled under the original protocol.)			
	*Loyola University of Chicago (LUMC) (Approval date: 10/00)		*Loyola University of Chicago (LUMC) (Approval date: Fall, 2002)			
Sites w/ DOD/PCRP Approval	*The University of the West Indies, Kingston, Jamaica (Approval date: 3/2002)		Chicago Area		Kingston, Jamaica	Accra, Ghana
	LUMC		LUMC	MERCY	UWI	KORLE BU
Dates of data collection by site under original and amended protocols (Eligibility retro-active up to 12 months prior to being contacted and asked to enroll.)	Apr 10, 2000-Dec 31, 2002 (Eligibility retroactive to 1/1/00)		Jan 1, 2003 - Feb 28, 2003	-	-	-
No. Enrolled	94		10	0	0	0

ADMINISTRATIVE TASKS

TIMELINE:

May-October 2001: Project planning with Dr. Gepi-Attee of the University of Accra, Ghana
Original protocol approved by Mercy Hospital IRB.

October 23 – November 1: Ghana site visit; feasibility of implementing *amended protocol* directly
 assessed with Dr. Gepi-Attee. Dr. Richard Kwasi Gyasi is mentioned as the
 likely pathologist on the project.

December 17, 2001: *Amended protocol* submitted to LUMC IRB for approval.

January 16, 2002: *Amended protocol* fully approved by LUMC IRB.

PERIOD COVERED BY PROGRESS REPORT - YEAR 3

March, 2002: Implementation of original protocol at the University of the West Indies,
 Kingston, Jamaica approved by DOD; recruitment resumes at the
 University of the West Indies shortly thereafter.

April 9, 2002: *Amended protocol* and revised budget requests submitted to DOD for
 detailed review and approval.

April 30, 2002: Annual Progress Report submitted (Year 2).

May 13-15, 2002: Memorandum of Record for the approval of the *amended protocol* at each
 clinical site (Loyola, Ghana and Mercy Hospital) issued to PI by Mercy
 Swatson. (See appendix.)

May 16, 2002: *Amended protocol* submitted to Noguchi Institute IRB in Accra.

July 14, 2002: *Amended protocol* receives conditional approval by the Noguchi Institute.

September 5, 2002: Response to the Noguchi Institute review of *amended protocol* submitted.

September 17, 2002: Responses to Memorandum of Record for *amended protocol* submitted

September 20, 2002: Additional administrative requests for *amended protocol* received.

October, 2002: *Amended protocol* fully approved at Mercy Hospital.

October 24, 2002: *Amended protocol* fully approved by Noguchi.

October 30, 2002: Response to these requests sent to Robin Dillner.

Fall, 2002: *Amended protocol* at LUMC approved by DOD

December 18, 2002: Reply from Robin Dillner with a few additional requests for research sites
 in Ghana and in Jamaica.

December/January 2003: 12-month no-cost extension for the project requested and granted.

March 27, 2003: Recruitment in Jamaica ends.

DATA COLLECTION, BIOCHEMICAL & MOLECULAR STUDIES,
DATA ANALYSIS

PERIOD COVERED BY PROGRESS REPORT - YEAR 3

<i>TIMELINE:</i>	<i>CASE REGISTRY: Sociodemographical, clinical and pathological data</i>	<i>BIOCHEMICAL & MOLECULAR STUDIES:</i>
March 2002:	Hand Audit Checks: Each form reviewed for completeness and coherence. (PI)	Plasma and prostate tissue samples sent to Tuft Univ. for antioxidant and fatty acid measurements.
April 2002:	1) Verification and missing data collection (research assistance /project coordinator) 2) 2 nd Round Hand Audit Checks (PI)	Extracted DNA samples sent to Howard Univ. for genotyping of candidate prostate cancer genes (variants of 5- alpha reductase, androgen receptor microsatellite and selected cytochrome P450 proteins).
May 2002:	Case registry form data entered using the double-keyed method. (RA/PC/PI)	1) Genotyping completed. 2) Raw data reviewed and questionable results re-assayed. (PI/Molecular geneticist)
June 2002:	Data cleaning and analysis (PI)	1) Genotyping results certified. 2) Nutrient assays completed.
July 2002:		1) Genetic data entered, cleaned and association studies performed. (PI) 2) Raw nutrient data reviewed. (PI)
August 2002:		1) Nutrient results certified. 2) Comparative studies performed on prostate tissue.
September 2002:		Database integrating demographic, clinical, pathological, nutritional and genetic data created. (PI)

RESULTS DISSEMINATION ACTIVITIES

TIMELINE:

PERIOD COVERED BY PROGRESS REPORT - YEAR 3

September 2002:	5 Scientific abstracts written (3 by PI, 2 by Jamaican collaborators) and circulated to current and future collaborators (including Drs. Terry Mason and John Cudecki of Mercy Hospital) for comments and revisions. (PI)
October 2002:	1 abstract submitted to the American Urological Association (AUA). (PI)
November 2002:	1) 2 abstracts submitted to the American Association for Cancer Research (AACR). (PI) 2) Jamaican case series paper revised and resubmitted to the <i>West Indian Medical Journal</i> . (Jamaica)
December 2002:	1) 2 abstracts submitted to the American Society of Clinical Oncology (ASCO). (Jamaica) 2) Consensus grading paper extensively revised and resubmitted to the journal <i>The Prostate</i> . (PI)
January 2003:	AACR abstracts accepted for poster presentations.
March 2003:	ASCO abstracts accepted (1 poster, 1 publication only).
April 2003:	Consensus paper accepted by the journal <i>The Prostate</i> .
June 2003	Poster presented at ASCO's annual meeting in Chicago (Jamaica/PI)
July 2003	Posters (2) presented at the AACR's annual meeting in Washington, DC (originally scheduled for Toronto in April, but cancelled due to the SARS epidemic). (PI)

II. BODY

Approved Statement of Work

Task 1. "Provide reliable recruitment of incident cases in region."

- a. Create consortia of urologist and pathologists in each of three regions: West Africa (Accra, Ghana), Jamaica and Chicago, IL.
- b. Develop incident case recruitment strategies appropriate for each research site, with the goal of *soliciting participation* of 75% of newly diagnosed cases per site (60-120/region) per year.
- c. To help meet our enrollment target for the Chicago-area, extend recruitment of eligible African-American men to Mercy Hospital-affiliated private practice.

We have been able to establish robust (ie., productive and reliable) recruitment strategies at our original research sites in Chicago and Jamaica, where solicitation and participation rates (using all eligible cases as the denominator) exceed 81% and 66%, respectively, in each site. The amended protocol was approved by Mercy Hospital's IRB in October of 2002. However, the Mercy Hospital site did not receive final approval by HSRRB before case enrollment in Chicago area ended a few months later. Also, we have not been able to begin enrolling patients at Korle Bu Teaching Hospital of the University of Ghana Medical School in Accra. This is the case despite 18 months (May 2001 thru November 2002) of great interest and cooperation on the part of the local PI and his institution and approval of the amended protocol in November of 2002. Shortly after the protocol was approved by the hospital's IRB (The Noguchi Institute), Dr. Gepi-Attee informed us that he would be out of the country on holiday until January 2003. Since mid-January, we attempted to contact him many times (including calls at his office and cell phone number) but to no avail. So, Dr. Gepi-Attee has effectively dropped out of sight. This is a puzzling development, and we have no explanation, just speculation (illness, institutional politics and/or other research opportunities). Trying to obtain HSRRB approval in Dr. Gepi-Attee's absence at this point does not seem like a reasonable course to take. Therefore, we will not be able to complete recruitment of cases from West Africa.

Task 2. "Characterize each case using a common protocol."

- a. Convene pathologists for a review of the Gleason grading system and group reading of representative slide of cases diagnosed in each region.
- b. Level of agreement between pathologists will be monitored using a 25% random sample from each site to be circulated to each pathologist.
- c. Identify and monitor adherence to a common set of tumor and lymph node staging procedures.

Task '2a' and '2c' have been completed. As for task '2b', slides have been collected are ready for exchange between our pathologist in Jamaica and Chicago.

Task 3. "Create a centralized repository for serum, plasma, leukocytes and prostate tissue for biochemical and molecular studies"

- a.. Collect plasma, serum, and leukocytes on each case at the time of diagnosis, as well as fresh normal prostate tissue at the time of surgery from those undergoing radical prostatectomy.
- b. Collect height and weight at baseline, and follow-up data on symptoms, response to treatment, recurrence/progression, vital status and causes of death using a structured questionnaire.
- c. Process and store all patient specimens in a -70° C freezer Edward Hines, Jr. VA, in Building 1, Room C208.

Tasks 3a-3b have been completed.

Task 4. "Link case demographic, clinical and pathologic characteristics to corresponding tissue samples using a computerized database."

- a.. Establish a single computerized registry of demographic, clinic and pathologic data for cases recruited in each region.
- b. Combine tissue and registry data into a single electronic record, linking case registry information to corresponding tissue samples using their unique barcode identification number.

A centralized repository for serum, plasma, leukocytes and prostate tissue has been created, and integration with case registry data (demographics, clinical and pathological findings) was completed in September 2001.

Task 5. . Pilot Studies: Conduct comparative studies of genes, nutrition and histopathologic markers of prognosis.

Results of our pilot studies have now been presented at major national scientific meetings (American Association for Cancer Research, American Society of Clinical Oncology). Dr. Clement Adebmowo (a surgical oncologist at the University of Ibadan who is presently a doctoral candidate in Epidemiology at Harvard University) generously donated data and samples from 49 cases enrolled in a separate study in Ibadan for comparisons involving West Africans. Abstracts and poster presentations are included in the appendix. Key findings (excepted from abstracts):

1. In a comparison of demographic and clinical characteristics between blacks in Chicago and Jamaican, "there were no significant differences in [mean age, marital status and tobacco use] between the two groups." However, Chicago blacks had a lower mean PSA (26.2 vs. 81 ng/ml, $p=0.0026$) and median PSA (9 vs. 20 ng/ml, $p<0.001$); a [slightly] higher mean Gleason sum (7.0 vs. 6.8, $p=0.0152$), and a higher likelihood of having localized disease..." Moreover, "there were

no significant difference in time to treatment, but Jamaicans had a greater likelihood of receiving hormone therapy, consistent with the higher incidence of extraprostatic disease in this group."

2. The central two assumptions in this research design are that blacks in America, the Caribbean and in West Africa share a common genetic background but reside in different environmental settings. To test the latter assumption more directly as well as identified possible shared environmental risk factors, we compared concentrations of tocopherols, carotenoids and retinol in the prostates of men undergoing radical prostatectomy for clinically localized prostate cancer. "Concentrations were significantly higher in Jamaicans compared to Chicagoans for tocopherols (42.5 vs. 29.2 nmol/g, $p=0.035$ and 8.5 vs. 5.9 nmol/g, $p=0.0024$ for alpha tocopherol [vitamin E] and gamma tocopherol, respectively), carotenoids (532 vs. 288.4 pmol/g, $p=0.0274$, 182.4 vs. 109.5 pmol/g, $p=0.0062$, 547.2 vs. 76.6 pmol/g, $p<0.001$, 499.4 vs. 272.2 pmol/g, $p=0.20$, for lutein, beta-cryptoxanthin, alpha and beta-carotene, respectively, and retinal (829.9 vs. 333.6 pmol/g, $p=0.006$)." However, "mean levels of lycopene were not statistically different (189.9 vs. 297.8 pmol/g, $p=0.3993$ for Jamaicans and Chicagoans, respectively."

We concluded that these preliminary results support a possible etiopathogenic role for lycopene in prostate cancer disease.

3. We also compared the fatty acid composition of prostate tissue of Jamaican blacks to that of Chicago blacks. In Jamaica blacks, "the concentration of total saturated fatty acid was lower (39 vs. 42.4%, $p<0.01$), that of omega-3 fatty acid and the ratio of omega-3 to omega-6 fatty acids were higher (4.7 vs. 2.6%, $p<0.005$; 0.54 vs. 0.30, $p<0.02$, respectively) than in prostate from [Chicago] blacks." Of note, the ratio of omega-3 to omega-6 fatty acid has been suggested to be a more important indicator of prostate cancer risks (incidence and severity).
4. We compared frequencies of the V89L variant of steroid 5-alpha reductase type II (SRD5A2) – the enzyme that converts testosterone to its active metabolite, dihydrotestosterone, and serves as the enzymatic target for the 5-alpha reductase inhibitor, finasteride – and characteristics of the androgen receptor (AR) CAG repeat sequence in cases Chicago, Jamaica and Nigeria. A sample of white Americans cases from Chicago served as an additional reference group. "The V89L variant, which correlates with reduced androgen activity, was significantly more prevalent in Chicago and Jamaican blacks than in Nigerians ($p<0.001$)." There were "no significant differences between Nigerians and Chicago whites in the distribution of genotypes ($p=0.8194$) even though there were significant ($p<0.001$) differences in stage at presentation." However, among black cases, a decline in the prevalence of variant 'L' allele across sites paralleled the decline in proportion of localized cases in each site."

With respect to the AR CAG repeat sequence, were shorter lengths associate with increased androgen activity, lengths "tended to be [different] among blacks across sites ($p=0.02$)." However, "[lengths] were generally shorter among blacks compared to whites ($p=0.0035$ to <0.001)."

We concluded that expression of any prostate cancer phenotypes that may associate with these genetic variants in US blacks and whites is likely context dependent.

5. Given the findings described above, we took a closer look at the relation between the V89L variant to cancer stage among blacks across sites. Among the 156 subjects available (38 Chicagoans, 76 Jamaicans and 42 Nigerians), "advanced cancers tended be underrepresented among homo and heterozygous V89L mutants in Chicago and Jamaica ($p=0.0388$ and 0.1138 , respectively), but not in Ibadan ($p=0.49$).\" However, \"after controlling for age and site, the V89L variant was significantly associated with a lower risk of advanced prostate cancer across sites (vs. wildtype, Odds Ratio [95% confidence interval] = 0.74 [$0.22, 2.53$] and 0.27 [$0.08, 0.96$] for VL and LL, respectively, p trend= 0.01).\" Information on PSA or tumor differentiation was missing for 15 of the 42 subjects from Ibadan. Consequently, neither variable was incorporated into the final model.

We concluded that functional mutations involving SRDRA2, such as the V89L mutation, may play a role in prostate cancer disease severity in black men. Furthermore, the role may be influenced by environmental and social settings.

III. KEY RESEARCH ACCOMPLISHMENTS:

The purpose of this research is to develop the infrastructure for comparative studies of prostate cancer among blacks who reside in contrasting environmental settings. The key accomplishments since our last annual report are as follows:

- Establishment of an epidemiological research infrastructure (ie., accessible populations, clinical resources and data collection methods) to support unified measurement of exposure and prostate cancer disease in Chicago, Illinois and Kingston, Jamaica.
- Completion of biochemical and genetic studies on just over 40% of subjects enrolled.
- Creation of a clean computerized database that links the demographical, clinical and pathological characteristics of each case to corresponding archived tissue specimens and the results of nutritional and genetic measurements performed on those specimens.
- Completion of statistical comparisons of *i*) demographical, clinical and pathological characteristics of cases from Chicago, Kingston and West Africa, *ii*) levels of antioxidants (tocopherols, carotenoids and retinol) and fatty acids in serum and prostate tissue in cases diagnosed in Chicago and Jamaica, *iii*) and have performed association studies between variants of genes involved in androgen metabolism and clinical stage of prostate cancer within and across cases from Chicago, Jamaica and West Africa.
- Finally, our first methods paper was accepted to the journal The Prostate, and initial findings from our pilot studies have been presented at national meetings and/or published in their proceedings.

IV. REPORTABLE OUTCOMES: Since the Study's Inception

A. Manuscripts:

1. Accepted or in-press –

Freeman VL, Coard KCM, Wojcik E, Durazo-Arvizu R. Use of the Gleason system in international comparisons of prostatic adenocarcinomas in blacks. *Prostate* November 2003 (in-press)

2. Under review –

Coard KCM, **Freeman VL**. Gleason grading of prostate cancer: level of concordance between pathologists at the University Hospital of the West Indies. *West Indian Medical Journal*

B. Abstracts:

1. **Freeman, VL**, Coard, K, Ogunbiyi, O, Wojcik, EM. Gleason scoring system: high level of agreement between pathologist from three countries. Proceedings of the United States and Canadian Academy of Pathology. *Lab Invest* Volume 81, pg. 108A, #624, January 2001
2. Wojcik, EM, Coard, K, **Freeman, VL**. Prostate cancer in African Americans and Jamaicans. Proceedings of the United States and Canadian Academy of Pathology. *Lab Invest* Volume 81, pg. 128A, #743, January 2001
3. Bennett FI, **Freeman VL**, Coard K, Aiken W, Tulloch T, Forrester T, Panton B, Flanigan R. Fatty acid composition of prostatic tissue from blacks in Jamaica and Chicago. *Proceedings of the American Society of Clinical Oncology*, Volume 22, #1658, May 2003
4. Aiken W, Tulloch T, **Freeman V**, Bennett F, Coard K, Panton P, Kittles R, Mason T, Flanigan R. Differences in Patient Characteristics in Black Men with Prostate Cancer from Jamaica and Chicago. *Proceedings of the American Society of Clinical Oncology*, Volume 22, #1764, May 2003.
5. **Freeman VL**, Kittles RA, Adebamowo A, Bennett F, Tullock T, Aiken W, Coard KCM, Panton B, Cudecki JJ, Mason T, Flanigan RC, Sylvester N. Steroid 5-alpha reductase type II V89L substitution and risk of advanced prostate cancer in black men from Nigeria, Jamaica and Chicago. *Proceedings of the American Association for Cancer Research*, Volume 44, 2nd ed., #3613, July 2003.
6. **Freeman VL**, Kittles RA, Bennett F, Aiken W, Tullock T, Coard KCM, Panton B, Adebamowo A, Mason T, Cudecki JJ, Flanigan RC, Sylvester N. Steroid 5-alpha reductase type II V89L variant frequencies and androgen receptor CAG microsatellite lengths among black men with prostate cancer from Nigeria, Jamaica and Chicago, Illinois. *Proceedings of the American Association for Cancer Research*, Volume 44, 2nd ed., #3614, July 2003.

C. Presentations:

- POSTERS -

1. 90th Annual Meeting of the United States and Canadian Academy of Pathology (USCAP), Atlanta, GA, USA. "Gleason scoring system: high level of agreement between pathologist from three countries" March 5, 2001.
2. 90th Annual Meeting of the United States and Canadian Academy of Pathology (USCAP), Atlanta, GA, USA. "Prostate cancer in African Americans and Jamaicans." March 5, 2001
3. 2003 Annual Meeting of the American Society of Preventive Oncology (ASCO), Chicago, IL. Fatty acid composition of prostatic tissue from blacks in Jamaica and Chicago. June 1, 2003.
4. American Association for Cancer Research (AACR), 94th Annual Meeting, Toronto, Canada: "Steroid 5-Alpha Reductase Type II V89L Substitution and Risk of Advanced Prostate Cancer in Black Men From Nigeria, Jamaica and Chicago." April 7, 2003. (Rescheduled for July 12, 2003, Washington, DC.)
5. American Association for Cancer Research (AACR), 94th Annual Meeting, Toronto, Canada: "Steroid 5-Alpha Reductase Type II V89L Variant Frequencies and Androgen Receptor CAG Microsatellite Lengths Among Black Men With Prostate Cancer From Nigeria, Jamaica and Chicago, Illinois." April 7, 2003. (Rescheduled for July 12, 2003, Washington, DC.)

D. Tissue Repository

Study site:		CHICAGO	KINGSTON		IBADAN	Total	
Enrollment eligibility dates		1/1/00 - 2/28/03	1/1/00 - 9/1/00	3/1/01- 3/19/02 ¹	3/30/02- 3/27/03	1/17/00- 9/1/00	
Recruitment periods		4/10/00- 2/28/03	5/22/00 - 9/1/00	-	3/30/02 3/27/03	4/17/00- 9/1/00	
(No. of weeks of recruitment)		(150)	(35)	(0)	(52)	(30)	
SUBJECTS	No. Eligible	156	74	?	132	49	362+
	No. Solicited (% eligible)	138 (88.5%)	61 (82.4%)	40	108 (81.8%)	28 (57.1%)	375
	No. Enrolled (% solicited)	104 (75.4%)	61 (100%)	40	105 (97.2%)	24 (85.7%)	334 (89.1%)
TISSUE ELEMENTS	Leukocytes	99	59	40	105	0	303
	Plasma/serum	98	59	0	105	0	262
	Prostate tissue	16	12	0	16	0	44

¹Incident cases occurring during this period were eligible for enrollment but were not solicited until HSRRB approval was granted 3/02. Forty of these cases were eventually solicited and enrolled after approval was granted.

²Pretreatment plasma and serum could not be obtained since all of the 40 cases had already been treated.

V. CONCLUSIONS:

Developing the infrastructure needed for comparative studies of prostate cancer among blacks who reside in contrasting environmental settings is feasible. The assumption that Chicago and Kingston differ in terms of environmental exposures (in this case, dietary exposures) is likely correct. However, our data in support of a shared genetic background based on frequencies distributions of the candidate prostate cancer genes we studied is less consistent.

Our data suggest that lycopene plays an etiopathogenic role in prostate cancer disease risk in both the US and in Jamaica. Also, inheriting the V89L variant of 5-alpha-reductase associates with a lower risk of advanced prostate cancer in multivariate analysis. This association is plausible given the physiological consequences of the V89L mutation and recent evidence from a large clinical trial linking inhibition of 5-alpha-reductase activity with a lower risk of incident prostate cancer (1).

Importance and/or implications: In addition to demonstrating the feasibility of international comparative studies of prostate cancer across different black populations, this work sets the stage for new studies examining potential gene-environment interactions. Research of this kind can, in turn, help us disentangle the relative roles of heredity and the environment in the development and prognosis of prostate cancer. These insights may be particularly helpful to those at highest risk for developing and dying from prostate cancer such as African-Americans.

The remainder of the 12-month no-cost extension will be used to prepare and submit manuscripts and research grant applications based on the results of our work.

This report has been respectively submitted by



Vincent Freeman, MD, MPH

Principal Investigator

Assistant Professor

Department of Medicine

Loyola University of Chicago

VI. REFERENCES:

1. Thompson IM, Goodman PJ, Tangen CM, et al. The influence of finasteride on the development of prostate cancer. *NEJM* 2003;349:215-24.

VII. APPENDICES:

1. Manuscript entitled, "Use of the Gleason system in international comparisons of prostatic adenocarcinomas in blacks." (Freeman et al.)
2. Agenda for Chicago-Kingston conference call of December 12, 2002
3. Table of patient characteristics
4. Abstracts and poster presentations

APPENDICES

Manuscript entitled, "Use of the Gleason system in international comparisons of prostatic adenocarcinomas in blacks." (Freeman et al.)

Agenda for Chicago-Kingston conference call of December 12, 2002

Table of patient characteristics

Abstracts and poster presentations

**USE OF THE GLEASON SYSTEM IN INTERNATIONAL COMPARISONS OF
PROSTATIC ADENOCARCINOMAS IN BLACKS**

Vincent L. Freeman, MD, MPH^{1,2,3}, Kathleen CM Coard, MD⁴, Eva Wojcik, MD⁵ and

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Short title: Gleason system in international studies

Abstract word count: 148

Text word count: 975

Tables: 1

Figures: 3

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DISCLOSURE STATEMENT

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Vincent L. Freeman, MD, MPH

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Eva Wojcik, MD

Ramon Durazo-Arvizu, PhD

Disclosure:

No relationships or conflicts of interest to disclose.

No relationships or conflicts of interest to disclose.

No relationships or conflicts of interest to disclose.

No relationships or conflicts of interest to disclose.

ABSTRACT

BACKGROUND: Comparisons of prostate cancer in blacks living in different countries can shed light on factors responsible for high rates of the disease among blacks in America. Since the prognostic value of the Gleason grading system is well established, we assessed agreement between pathologists in countries where black populations of the African Diaspora reside.

METHODS: Three genitourinary pathologists at hospitals in Nigeria, Jamaica and the US independently assessed sextant biopsies from 12 patients. Gleason sum and percentage involvement were recorded, and a percent-weighted average calculated. Agreement under different groupings was evaluated using the kappa statistic generalized to three raters.

RESULTS: Agreement was significant for individual sums ($\kappa=0.3317$, $p=0.0173$), sums grouped as well (2-4), moderately (5-6) and poorly differentiated (7-10) ($\kappa=0.2437$, $p < 0.0001$) and other groupings. Agreement between at least two raters was 91.7%-100%; complete agreement was 41.7%-66.7%.

CONCLUSIONS: The Gleason system is feasible and practical for international studies of prostate cancer among blacks from contrasting environments.

Key words: prostatic neoplasm, African Americans, prognosis

INTRODUCTION

Comparative of studies of prostate cancer disease among blacks who reside in contrasting environmental settings can shed light on the environmental and inheritable factors responsible for the high rates of the disease among blacks in the U.S. [1]. However, conducting such studies are complex, with standardized assessment of disease severity and prognosis posing particularly difficult challenges. Correlation between prostate cancer's diverse architectural and cytologic appearances and its wide-ranging biologic behavior is widely recognized [2-5]. Several different grading systems that group these appearances into prognostically relevant grades of prostate cancer have been proposed [6-10]. However, the architecturally based Gleason system is the one in most general use worldwide [10]. Its value for clinical prediction has been established in a greater number of patient-years follow-up than for any other system, criteria for assigning grade are clearly defined and relatively reproducible [10-14]. Indeed, the World Health Organization recommends that histologic grading using the Gleason system be used routinely in the prognostic evaluation of prostatic adenocarcinomas [15].

Therefore, the Gleason system should play an integral role in the prognostic stratification of prostatic adenocarcinomas in international comparisons of the disease. However, assigning Gleason sums is susceptibility to inter-individual variation, even between pathologists from the same institution. To examine the feasibility of using the Gleason system in this context, we assessed the level of agreement in the assignment of Gleason sums between pathologists practicing in three countries where black populations of the African Diaspora reside.

MATERIALS AND METHODS

Twelve slides of sextant biopsies positive for prostate cancer were randomly selected from archives at our institution. Three pathologists, one each from Kingston, Jamaica, Ibadan, Nigeria and the Chicago metropolitan area, were asked to participate. Three criteria for selection were used: 1) genito-urinary pathology was their subspecialty, 2) their practice was based primarily at an academic institution, 3) and they reviewed the majority of prostate tissue specimens collected at their institution. Slides of the 12 U.S. cases were circulated to the pathologists for independent histologic evaluation. Each was blinded to the question under study, and there was no advance didactical preparation. Gleason sum (GS) and percentage involvement of each positive core were recorded using a uniform scoring form, a percent-weighted average GS was calculated for each subject for each pathologist and sums rounded to the nearest integer. The Kruskal-Wallis statistic was used to compare mean sums, and agreement under three pre-determined groupings was evaluated using the kappa (κ) statistic generalized to three raters as described by Fleiss [16]. (See appendix.)

RESULTS

Table 1 compares the variability of Gleason sums assigned by each pathologist to the twelve cases. Although sums assigned by pathologist #3 demonstrated the greatest variation, mean sums between pathologists were not statistically different from one another ($p = 0.411$). Excluding a 'negative' biopsy result from pathologist 3 moved the mean sum (SD) to 6.35 (1.18) ($p = 0.684$).

Figures 1 thru 3 show the Gleason sums assigned to each case by each pathologist under various groupings. 'Complete agreement' was defined as all three pathologists assigning the case to the same category. For individual sums (Figure 1), complete agreement was observed in 5 of 12 cases (41.6%), and agreement between at least two pathologists was seen 11 of 12 cases (91.7%) ($\kappa = 0.3317$, $p = 0.0087$). Figure 2 shows pathologists' assignments when sums were grouped as well (GS 2 to 4) moderately (5 to 6) and poorly (7 to 10) differentiated. Complete agreement occurred in 8 of 12 cases (66.7%), and agreement between at least two pathologists was again observed in 11 of 12 cases ($\kappa = 0.2437$, $p < 0.0001$). When sums were grouped as well to moderately differentiated (2 to 6), GS=7 and poorly differentiated (8 to 10) (Figure 3), agreement between at least two pathologist occurred in 100% of cases, but complete agreement was observed in only half ($\kappa = 0.2761$, $p=0.0336$).

DISCUSSION

International variation in prostate cancer mortality suggests a causative role for environmental factors. Among black populations, available data seemed to support a gradient of risk with relatively low rates in Africa, intermediate rates in the Caribbean and the highest rates in America [17]. However, recent reports of comparable disease rates in these regions appear to dispute these historically accepted ranking and suggest a possible role for inheritable or shared lifestyle factors [18-20]. International comparisons of prostate cancer disease in these populations can play an important role in helping to disentangle the contributions of the environment and genetics to prostate cancer disease in general. Therefore, having robust and reproducible markers of prognosis with which to make such epidemiologic comparisons would be essential. Our results suggest that use of the Gleason system is not only feasible but is practical for this purpose. The level of agreement between the three pathologists based on various measures was generally high. However, the finding of full concordance in only six of the twelve cases when sums were grouped as < 7 , 7 and > 7 suggests a need for approaches that help better distinguish between Gleason sums of 6 and 7. The Internet and web-based strategies hold considerable promise in this regard [21].

The strengths of this study included blinding pathologists to the question under study, lack of advanced didactical preparation on their part and independent assessment. Although slides were selected at random, a potentially important limitation was the narrow spectrum of sums evaluated. While it may have reflected the average distribution of histopathologic findings, not all possible sums or combinations were evaluated. This could have enhanced performance, thus overestimating agreement.

CONCLUSIONS

The Gleason system for the routine prognostic evaluation of prostate cancer appears to be both feasible and practical for international comparative studies of the disease in black populations of the African Diaspora. Strategies that help distinguish between sums of 6 and 7 could greatly enhance the validity of the Gleason system for standardized disease assessment under this study design.

APPENDIX

Calculation of Kappa Statistic: The use of kappa and weighted kappa is usually restricted to the case where both the number of raters is two and where the same two raters rate each subject. The method used to calculate kappa in this setting was based on that described by Fleiss (15). This method considers the for the case of more than two raters and for the case where the raters judging one subject are not necessarily the same as those judging another.

Let N = the total number of subjects (cases), n = number of raters (pathologists) per subject, k = number of categories (grades), n_{ij} = number of raters who assigned the i^{th} subject to the j^{th} category and p_j = the proportion of all assignments made to the j^{th} category ($= \sum n_{ij}/Nn$). The level of agreement among 'n' pathologist for the i^{th} subject can then be indexed by the proportion of agreeing pairs out of all the $n(n - 1)$ possible pairs of assignments. This proportion is:

$$P_i = \frac{1}{n(n-1)} \sum_{j=1}^k n_{ij}(n_{ij} - 1) = \frac{1}{n(n-1)} \left(\sum_{j=1}^k n_{ij}^2 - n \right)$$

The overall extent of agreement is then measured by the mean of P_i 's :

$$P = \frac{1}{Nn(n-1)} \sum_{j=1}^k n_{ij}(n_{ij} - 1) = \frac{1}{Nn(n-1)} \left(\sum_{i=1}^N \sum_{j=1}^k n_{ij}^2 - Nn \right)$$

Let the mean of the P_i 's = .60. This result is interpreted as follows: Let a case be selected at random and graded by a randomly selected pathologist. If the case were to also be graded by a randomly selected pathologist, the second pathologist would agree with the first for 60% of the time. However,

some degree of agreement would be expected solely on the basis of chance. In fact, if the pathologists made their assignments purely at random, one would expect the mean proportion of agreement to be:

$$P_{J \text{ expected}} = \sum P_j^2 = 0.3765$$

The quantity $1 - P_{\text{expected}}$ measures the degree of agreement attainable over and above what would be expected by chance. Kappa (κ) is the degree of agreement actually attained in excess of chance is calculated as follows:

$$\kappa = \frac{P - P_{\text{expected}}}{1 - P_{\text{expected}}}$$

Which is a normalized measure of overall agreement corrected for the amount expected by chance. Since this normalized estimate of κ follows a normal distribution, we can test the following null hypothesis to evaluate whether the normalized estimate of agreement between the pathologists is significant:

$$H_0: \frac{\kappa}{\text{(Standard error)}} < 1.96,$$

This is equivalent to the hypothesis of “no agreement”. The standard error of κ (S.E. (κ)) is equal to the square-root of the variance of κ ($\text{Var}(\kappa)^{1/2}$). The variance of κ under the hypothesis of “no

agreement" beyond chance is approximately equal to:

$$\text{Var}(\kappa) = \frac{2}{Nn(n-1)} \times \left[\sum P_i^2 - (2n-3) \left(\sum P_i^2 \right)^2 + 2(n-2) \left(\sum P_i^3 \right) \right] (1 - \sum P_j^2)^2$$

If $\kappa / (\text{S.E.}) > 1.96$, then the agreement between pathologists is significantly greater than you would expect by chance alone.

ACKNOWLEDGMENT: The work was supported by research grants from the U.S. Department of Defense (DAMD17-00-1-0029) and the Department of Veterans Affairs (RCD 97-317). We also thank Dr. Olufemi Ogunbiyi of the University of Ibadan, Nigeria for his participation in this study.

REFERENCES

1. Kittles RA, Chen W, Pangulari RK, Ahaghotu C, Jackson A, Adebamowo CA, Giffin R, Williams T, Ukoli F, Adams-Campbell L, Kwagyan J, Isaacs W, Freeman V, Dunston, GM. CYP3A4-V and prostate cancer in African Americans: causal or confounding association because of population stratification. *Hum Genet* 2002;110:553-560.
2. Galle MPW, Ten Kate JW, Mulder GH. Histologic grading of prostatic carcinoma in prostatectomy specimens. *Br J Urol* 1990;65:368-375.
3. Schroeder FH, Bloom JH, Hop WC, Mostofi FK. Grading of prostatic carcinoma: I. An analysis of the prognostic significance of single characteristics. *Prostate* 1985;6:81-100.
4. Grahack JT, Assimos DG. The prognostic significance of tumor grade and stage in patients with carcinoma of the prostate. *Prostate* 1983;4:13-433.
5. Gleason DF. Histologic grading of and staging of prostatic carcinoma. In: Urologic Pathology: The Prostate. Tannenbaum M, (Ed.) Phila 1977. pp 171-197.
6. Utz DC, Farrow GM. Pathologic differentiation and prognosis in and prognosis in prostatic carcinoma. *JAMA* 1969;209:1701.
7. Mostofi FK. Grading of prostatic carcinoma. *Cancer Chemother Rep (part I)* 1975;59:111-117.
8. Harada M, Mostofi FK, Corle DK, Byar DP, Trump BF. Preliminary studies of prognosis in cancer or the prostate. *Cancer Treat Rep* 1977;61:223-225.
9. Brawn P, Ayala AG, Von Eschenbach AC, Hussey DH, Johnson DE. Histologic grading study of prostate adenocarcinoma: The development of a new system and comparison with other methods-a preliminary study. *Cancer* 1982;49:525-532.

10. Gleason DF, Mellinger GT, The Veterans Administration Cooperative Urologic Research Group. Prediction of prognosis for prostatic adenocarcinoma and combined histologic grading and clinical staging. *J Urol* 1974;111:58-64.
11. Sogani P, Israel A, Lieberman P, Lesser ML, Whitmore WF Jr. Gleason grading of prostate cancer: A predictor of survival. *Urology* 1985;25:223-227.
12. Nemoto R, Uchida K, Harada M, Koiso K, Abe R, Kato T. Experience with Gleason histopathologic grading of prostate cancer in Japan. *Urology* 1987;30:436-440.
13. Gardner WA, Coffey D, Karr JP, Chiarodo A, Epstein J, McNeal JE, Miller G. A uniform histopathologic grading system for prostate cancer. *Human Pathol* 1988;19:119-120.
14. Murphy GP, Whitmore WF. A report of the workshops on the current status of the histologic grading of prostate cancer. *Cancer* 1979;44:1490-1494.
15. Bostick DG, Foster CS. Predictive factors in prostate cancer: current concepts from the 1999 College of American Pathologists Conference on Solid Tumors Prognostic Factors and the 1999 World Health Organization Second International Consultation on Prostate Cancer. *Semin Urol Oncol* 1999;17:222-72.
16. Fleiss JL. Measuring nominal scale agreement among many raters. *Psych Bull* 1971;76:378-82.
17. Muir CS, Nectoux J, Staszewski J. The epidemiology of prostatic cancer. Geographical distribution and time-trends. *Acta Oncol* 1991;30:133-40.
18. Osegebe DN. Prostate cancer in Nigerians: facts and nonfacts. *J Urol* 1997;157:1340-43.
19. Glover FE, Coffey DS, Douglas LL, Cadogan M, Russell H, Tulloch T, Baker TD, Wan RL, Walsh PC. The epidemiology of prostate cancer in Jamaica. *J Urol* 1998;159:1984-87.

20. Bunker CH, Patrick AL, Konety BR, Dhir R, Brufsky AM, Vivas CA, Becich MJ, Trump DL, Kuller LH. High prevalence of screening-detected prostate cancer among Afro-Caribbeans: the Tobago Prostate Cancer Survey. *Cancer Epidemiol Biomarkers Prev* 2002;11:726-9.
21. Kronz JD, Silberman MA, Allsbrook WC, Baskacky SI, Burks RT, Cina SJ, Mills SE, Ross JS, Sakr WA, Tomaszewski JE, True LD, Ulbright TM, Weinstein MW, Yantiss RK, Young RH, Epstein JI. Pathology resident's use of a Web-based tutorial to improve Gleason grading of prostate carcinoma on needle biopsies. *Hum Pathol* 2000;31:1044-50.

Table 1. Descriptive statistics

Pathologist	1	2	3	p-value
Gleason sum range ¹	6 - 7.23	6 - 8	0 - 8.11	
Mean Gleason score (SD)	6.38 (0.14)	6.67 (0.18)	5.82 (0.62)	0.411

¹Gleason score (GS) calculated as the %-of-core-weighted mean GS.

LEGENDS

FIGURE 1. Gleason sum (GS) assignments for each case. GS = 0 for biopsies (N=1) interpreted as negative by a pathologist. Kappa (κ) = $P_0 - P_E / 1 - P_E$, where P_0 and P_E is the observed and expected proportion of agreement, respectively.

FIGURE 2. Gleason sum (GS) assignments for each case with sums grouped into 2-4, 5-6 and 7-10, corresponding to well, moderated and poorly differentiated, respectively. GS = 0 for biopsies (N=1) interpreted as negative by a pathologist. Kappa (κ) = $P_0 - P_E / 1 - P_E$, where P_0 and P_E is the observed and expected proportion of agreement, respectively.

FIGURE 2. Gleason sum (GS) assignments for each case with GS = 7 considered separately. GS = 0 for biopsies (N=1) interpreted as negative by a pathologist. Kappa (κ) = $P_0 - P_E / 1 - P_E$, where P_0 and P_E is the observed and expected proportion of agreement, respectively.

FIGURE 1.

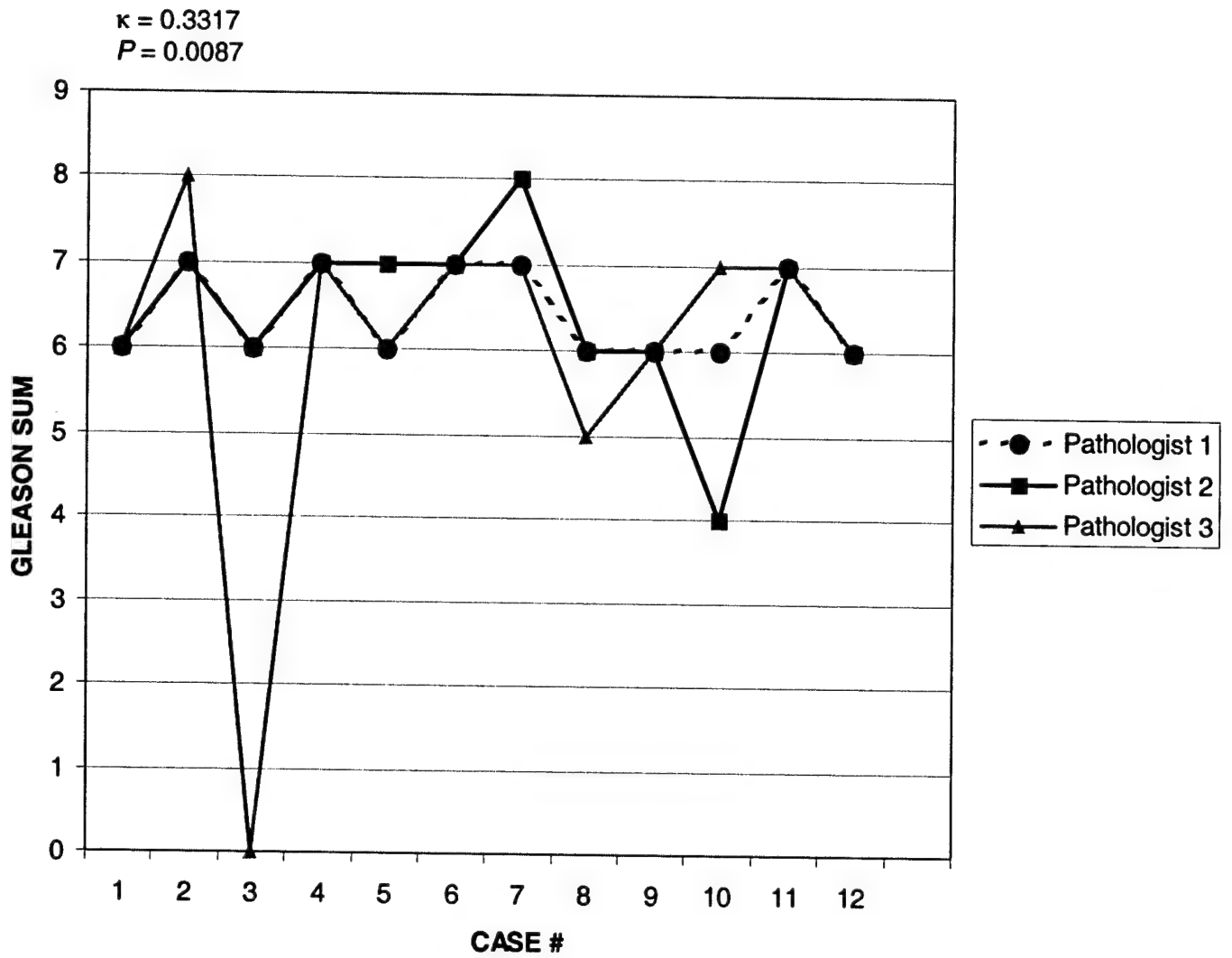


FIGURE 2.

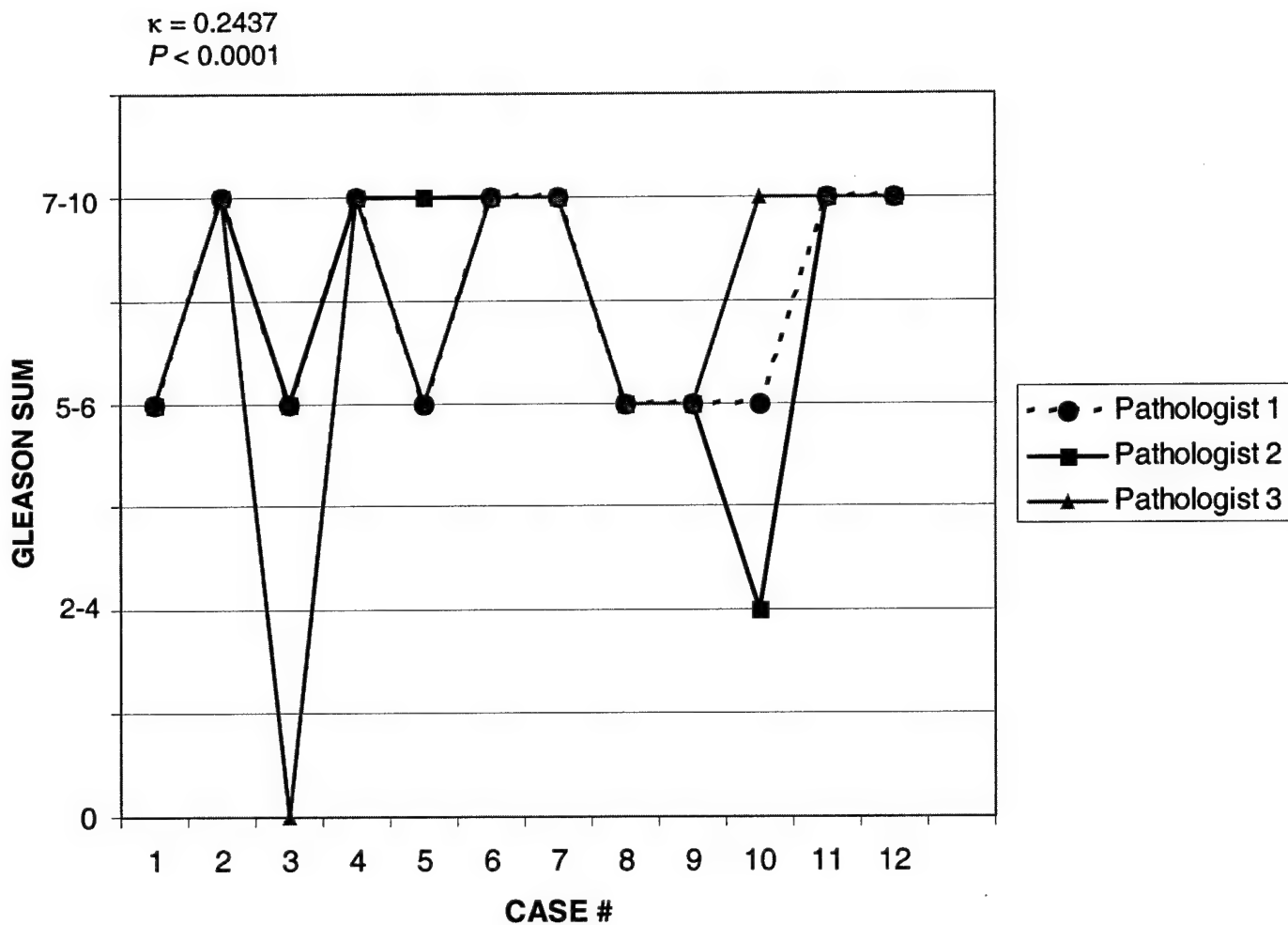
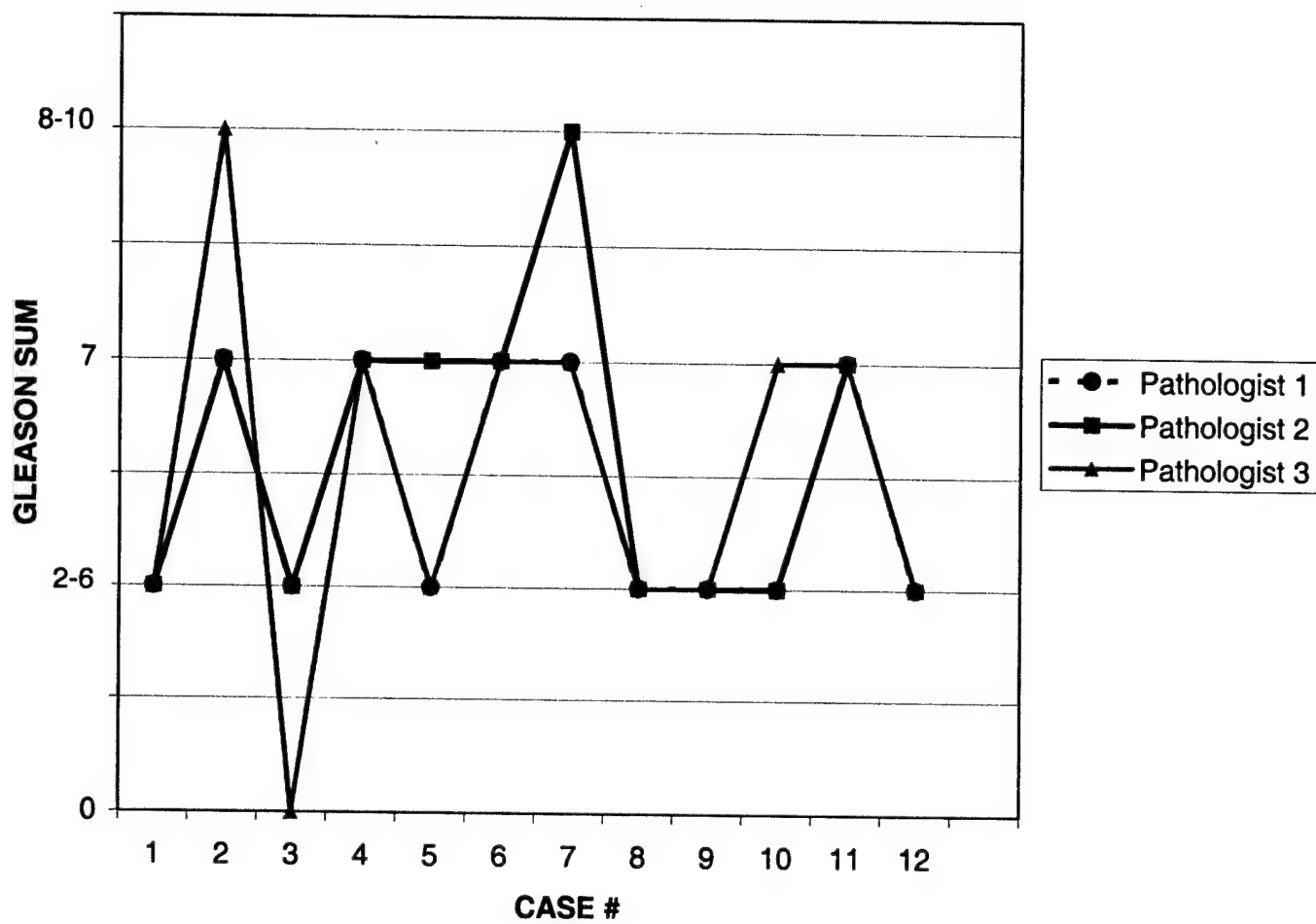


FIGURE 3.

$\kappa = 0.2761$
 $P < 0.0336$



PROSTATE CANCER IN WEST AFRICANS (GHANAIS), JAMAICANS AND US BLACKS
Chicago-Jamaica Conference Call (12/12/02)

AGENDA

I. ENROLLMENT

- A. Chicago
- B. Jamaica

II. ADMINISTRATIVE ISSUES

A. Ghana

- 1. Pathologist identified in Ghana:

Dr. Richard Gyasi
Department of Pathology
University of Ghana Medical School
P.O. Box 4236, Accra Ghana
Tel. 00233 21 661-302 & 675-577
rkg539us@yahoo.com

Dr. Gyasi is a Sr. Lecturer and Specialist Pathologist at Korle Bu Teaching Hospital in Accra.

- 2. Local IRB approval was granted in November; DOD's pending.
- 3. Visit planned for June 19-26, 2003.

B. Chicago

- 1. Pathologist to replace Eva identified: Dr. Sherri Yong.

C. No-cost Extension of Project Past March 2003.

III. Review of Literature (2001-2002)

IV. Data Dissemination

A. Abstracts Submitted (N=3):

TO AUA (American Urologic Association):

- 1. "Antioxidants in Black Men With Localized Prostate in Jamaica and Chicago: prostatic concentrations are significantly higher in Jamaicans than in Chicagoans Except Lycopene"
Freeman, Bennett, Aiken, Tulluck, Coard, Meydani, Forrester and Flanigan.

TO AACR (American Association for Cancer Research):

- 1. "Steroid 5-alpha Reductase Type II V89L Variant Frequencies and Androgen Receptor CAG Microsatellite Lengths Among Black Men with Prostate Cancer From Nigeria, Jamaica and Chicago."
Freeman, Kittles, Bennett, Aiken, Tullock, Coard, Panton, Adebamowo, Mason, Cudecki, Flanigan and Sylvester.

2. "Steroid 5-alpha Reductase Type II V89L and Risk of Advanced Prostate Cancer in Black Men From Nigeria, Jamaica and Chicago."
Freeman, Kittles, Adebamowo, Bennett, Tullock Aiken, Coard, Panton,, Mason, Cudecki, Flanigan and Slyvester..

B. Abstracts in to be submitted or in development (2):

TO ASCO (American Society of Clinical Oncology):

1. "Fatty acid composition of prostatic tissue from blacks in Jamaica and Chicago.
Bennett. (As of 11/19/02)

Consumption of dietary fats is one of the environmental factors thought to contribute to the wide variation in the incidence and mortality of prostate cancer in populations around the world (eg. mortality in Jamaican blacks is 54 per100 000 compared with in US blacks). Data on the association between prostate cancer saturated or unsaturated fats (intake or serum levels) are inconsistent. However, the ratio of different types of fats (unsaturated to saturated) or fatty acids (omega-3 to omega-6) to prostate cancer may be more important. Measurement of fat and fatty acid content of prostate tissue from populations with different rates of prostate cancer may help to clarify the association. The fatty acid content of prostatic tissue removed from Jamaican and US blacks at open prostatectomy was therefore determined. In Jamaican blacks the concentration of saturated fatty acids was lower (42.4 v 39.0% $p < 0.001$), that of omega-3-fatty acids and the ratio of omega-3 to omega-6 fatty acids were higher (2.6 v 4.7%, $p < 0.005$; 0.30 v 0.54, $p < 0.02$ respectively) than in prostatic tissue from American blacks. There were no differences in the concentrations of total saturated, mono-unsaturated and polyunsaturated fats, oleic and α -linolenic acids or in the ratio of polyunsaturated to saturated fatty acids. Among other mechanisms, fats may contribute to prostate cancer risk by increasing the level of endogenous androgens or providing substrate for the generation of free radicals. Conversely, increased omega-3- fatty acids and a high ratio of omega-3 to omega-6 fatty acids may lower the risk for prostate cancer by decreasing free radical formation. The finding of different levels of fats and fatty acids in prostatic tissue from US and Jamaican blacks suggest that these mechanisms may contribute to the difference in mortality from prostate cancer.

Franklyn, here are the references you asked for:

Rose DP, Connolly JM. Omega-3 fatty acids as chemopreventive agents. *Pharmacol Ther* 1999; 83:217

Simonsen N, et al. Adipose tissue omega-3 and omega-6 fatty acid content and breast cancer in the Euramc Study. *Amer J Epidemiol* 1998;147:342

2. Trevor and William.

C. Manuscripts.

1. Resubmitted to The Prostate (12/5/02):
 - a. Use of the Gleason system in International Comparisons of Prostatic Adenocarcinomas in Blacks. *Freeman, Coard, Durazo-Arvizu and Wojcik.*
2. Revision Pending
 - a. Eva's paper?
 - b. Kathy's paper
3. In development
 - a. Trevor's case-series paper
 - b. Franklyn's prostatic fatty acid paper.

V. Summary of 9th Prouts Neck Meeting: "Early Events in Prostate Carcinogenesis: Opportunities for intervention strategies and markers."

A. Pre-malignant lesions (PIN, PIA = prostatic inflammatory atrophy and PAH = proliferative atrophic hyperplasia)

B. Proteomic analyses of prostate cancer.

C. Genetic Alterations in prostate cancer development.

D. Environmental Influences.

New research model or paradigm: Environmental and lifestyle determinants of levels of markers for Inflammation -> angiogenesis -> invasion -> apoptosis in relations to disease severity.

E. Key technical issues:

1. Correlation studies (peripheral and/or intra-operative fat biopsies with prostate tissue nutrient concentrations.)
2. Tumor tissue for molecular studies.
3. BLISS

VII. Possible Visit (March 2003)

A. Manuscript Writing

B. Small and large grant development (Identifying sets of Specific Aims and factor focus).

C. NIH/NCI Grant deadline: October 1st, 2003 vs. February 1, 2004.

1. Chicago-Kingston comparison

Vincent Freeman, MD

TABLE 1. PATIENT CHARACTERISTICS

		Chicago	Kingston	Ibadan
Number enrolled		81	80	73
Age, yrs. Mean (\pm SD): [range]:		66.2 (8.3): [49.1-87.9]	68.8 (9.7): [50.5-92.7]	68.2 (9.7): [50.0-96.0]
Age distribution (%):	< 55 y	8.6	10.0	9.6
	55-64 y	40.7	22.5	24.7
	65-74 y	37.0	46.3	37.0
	≥ 75 y	13.6	21.3	28.8
Marital status (%):	Single	11.1	18.8	0
	Married or Widowed	79.0	77.5	95.9
	Other	9.9	3.8	4.1
Smoker (%):	Ever	57.5	65.0	48.0
	Current	12.5	11.3	48.0
Self-reported family history of prostate (%):		10.0	18.8	11.0
PSA Levels (%):	<4.0	8.6	2.5	61.6
	4.0-9.9	49.4	20.0	4.1
	10-19.9	25.9	25.0	4.1
	20-99.9	9.9	27.5	13.7
	100<	6.2	25.0	16.4
PSA (ng/ml). Mean (\pm SE)/Median: [range]:		26.2 (8.2)/9.0: [0.03-538]	81.0 (15.8)/20.0: [2.9-753]	125.5/59.0: [2-510] (n=31)
Mean Gleason sum:	Biopsy	6.6	7.0	Unavailable
	RP	7.0	6.8	
Differentiation (%):	Well (GS 2-4)	6.2	0	0
	Moderate (GS 5-6)	40.7	32.5	11.0
	Poorly or Undiff. (GS ≥ 7)	53.1	67.5	89.0
Organ-confined vs. Extraprostatic Disease (%):	Organ confined	76.5	51.3	13.7
	Extraprostatic	23.5	48.7	83.6
	Unstaged	0	0	2.7
Time to Treatment (days). Mean (\pm SE) /Median: [range]:		86 (12.5)/47 [0-791]	74 (7.3)/62 [0-334]	Insufficient data
1 st -Course Tx (%):	Organ-confined:			
	Observation	16.1	17.1	
	Radical Prostatectomy	40.3	34.2	
	Radiation	32.3	2.6	
	Hormones	1.6	34.2	
Extraprostatic:	Other	9.7	7.3	
	Observation	0	7.7	
	Radical Prostatectomy	52.6	2.6	
	Radiation	15.8	2.6	
	Hormones	10.5	71.8	
Unstaged:	Other	21.1	15.4	
	Hormones w or w/o Radiation	0	0	
		0	0	Insufficient data

Differences in Patient Characteristics in Black Men with Prostate Cancer from Jamaica and Chicago

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It is uncertain whether differences in prostate cancer incidence and mortality between African American men and White American men are due to differences in environmental exposures, ethnicity / genetic make-up and/or differences in access to health care. Comparisons of prostate cancer in Blacks who reside in different environments could enable the relative contributions of environmental exposures versus genetics, as well as access to health care to be determined. Two diverse populations of primarily African descent from different environments were compared - one North American, the other Caribbean. Incident cases of prostate cancer were identified at the two sites. Baseline demographic and clinical data as well as pretreatment blood samples for measurement of PSA were taken at the time of enrolment. The Gleason scores of patients who had radical prostatectomy were recorded. Demographic comparisons were made by Chi-Square analysis while mean PSA and Gleason scores were compared by 2 sample t-tests. 161 patients were identified, 81 from Chicago and 80 from Jamaica. There were no significant differences in demographic characteristics between the two groups. When compared to Jamaicans, Chicago Blacks had a lower mean PSA (26.2 vs 81 ng/ml, $p=0.0026$) and median PSA (9 vs 20 ng/ml, $p<0.001$); a higher mean Gleason sum (7.0 vs 6.8, $p=0.0152$), and a higher likelihood of having localized prostate cancer (76.5% vs 51.3%, $p=0.0008$). There was no significant difference in time to treatment, but Jamaicans had a greater likelihood of receiving hormone therapy, consistent with the higher incidence of extraprostatic disease in this group. Differences in clinical characteristics between men of African descent in environmentally disparate situations probably reflect differences in prostate cancer screening practices as well as awareness and access to health care, rather than biological or environmental differences influencing clinical presentation and tumor characteristics.

Key words: prostate cancer, clinical characteristics

Sponsor: name, address, telephone, fax, e-mail

Category: GUP-prostate cancer

Presentation preference: poster

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ANTIOXIDANTS IN BLACK MEN WITH LOCALIZED PROSTATE IN JAMAICA AND CHICAGO: PROSTATIC CONCENTRATIONS ARE SIGNIFICANTLY HIGHER IN JAMAICANS THAN IN CHICAGOANS EXCEPT LYCOPENE.

Vincent L. Freeman*, Hines, IL; Franklyn Bennett, Kingston, Jamaica; William Aiken, Kingston, Jamaica; Trevor Tullock, Kingston, Jamaica; Kathleen Coard, Kingston, Jamaica; Mohsen Meydani, Boston, MA; Terrence Forrester, Kingston, Jamaica; Robert C. Flanigan, Maywood, IL

Introduction and Objective: Comparative studies of prostate cancer among black men residing in contrasting environmental settings may shed light on causative and promoting exposures from the environment and the inheritable aspects of the disease that contribute to the excess prostate cancer incidence and mortality among African Americans compared to U.S. whites. Dietary intake of various antioxidants, especially lycopene, may influence prostate carcinogenesis. To learn more about the role of antioxidants from the environment, we compared concentrations of tocopherols, carotenoids and retinol in the prostates of men undergoing radical prostatectomy for clinically localized prostate cancer in Kingston, Jamaica and Chicago, Illinois.

Methods: Thirty-five men of West African ancestry (22 from Chicago, 13 from Kingston) awaiting radical prostatectomy for clinically localized prostate cancer were identified at Loyola University Medical Center, Hines VA Hospital (Chicago, Illinois) and the University of the West Indies, Mona (Kingston, Jamaica). Fresh, non-malignant tissue was collected from the peripheral zone of the prostate gland at the time of surgery. Concentrations of tocopherols (alpha and gamma carotenoids (alpha and beta-carotene, lutein, beta-cryptoxanthin and lycopene) and retinol were measured using high-performance liquid chromatography. The Wilcoxon rank sum test was used to compare mean prostatic concentrations in each site.

Results: Concentrations were significantly higher in Jamaicans compared to Chicagoans for tocopherols (42.5 vs. 29.2 nmol/g, $p=0.35$ and 8.5 vs. 5.9 nmol/g, $p=0.0024$ for alpha and gamma-tocopherol, respectively), carotenoids (532.0 vs. 288.4 pmol/g, $p=0.0274$, 182.4 vs. 109.5, pmol/g, $p=0.0062$, 547.2 vs. 76.6 pmol/g, $p<0.001$, 499.4 vs. 272.2 pmol/g, $p=0.020$, for lutein, beta-cryptoxanthin, alpha and beta-carotene, respectively) and retinol (829.9 vs. 333.6 pmol/g, $p=0.006$). However, mean levels of lycopene were not statistically different (189.9 vs. 297.8, $p=0.3993$ for Jamaicans and Chicagoans, respectively).

Conclusions: These results support a possible etiopathogenic role for lycopene in prostate cancer disease.

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Vincent L Freeman - No Disclosure Necessary

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Terrence Forrester - No Disclosure Necessary

Robert C Flanigan - No Disclosure Necessary

ABSTRACT

Key and composition of prostatic tissue from blacks in Jamaica and Chicago. Franklyn Bennett¹, Vincent Freeman², Kathleen Coard¹, William Aiken¹, Trevor Tulloch¹, Terrence Forrester¹, Barbara Pantoni¹, Mohsen Meydani³, Terry Mason⁴, John Cudecki⁴, Richard Flanigan². ¹Tropical Medicine Research Institute, University of the West Indies, ²Department of Medicine, Loyola University Stritch School of Medicine, Maywood IL, ³Vascular Biology Laboratory, USDA J-M Human Nutrition Research Center, Boston MA, ⁴Prairie Medical Associates, Mercy Hospital, Chicago IL.

Comparison of dietary fat intake and the ratio of omega-3 to omega-6 fatty acids in the diet and in the prostatic tissue of 31 men of African descent (Kingston, Jamaica) and 31 men of European descent (Chicago, IL). The mean age was 58.7 years (range 45-75). The mean body mass index was 26.5 kg/m² (range 21.5-31.5). The mean total fat intake was 100 g/day (range 70-130 g/day). The mean ratio of omega-3 to omega-6 fatty acids in the diet was 0.05 (range 0.02-0.10). The mean ratio of omega-3 to omega-6 fatty acids in the prostatic tissue was 0.05 (range 0.02-0.10). The mean ratio of omega-3 to omega-6 fatty acids in the prostatic tissue was significantly higher than the ratio in the diet (p < 0.05). The mean ratio of omega-3 to omega-6 fatty acids in the prostatic tissue was significantly higher than the ratio in the diet (p < 0.05).

Introduction

There is wide geographic and ethnic variation in the incidence of prostate cancer. Rates are highest in the US (104/100,000) and lowest in China (2/100,000). There is also wide variation within ethnic groups: US Blacks 238.9 compared with Jamaicans 56.4/100,000. Both genetic and environmental factors are thought to contribute to the development of prostate cancer and in populations of similar genetic make up, differences in incidence may be partly explained by differences in environmental exposure. Dietary fat intake is one important environmental exposure for which there is conflicting evidence of its role in the development of prostate cancer.

Objective

To determine, whether in subjects with similar genetic makeup but a different incidence of prostate cancer, there are differences in the fat and fatty acid content of prostatic tissue.

Methods

Fresh, non-malignant tissue was collected from the peripheral zone of the prostate gland of 31 men of West African ancestry (22 from Chicago, 9 from Kingston) who had radical prostatectomies for clinically localized prostate cancer at Loyola University Medical Center, Hines VA Hospital (Chicago, Illinois) and the University Hospital of the West Indies, (Kingston, Jamaica). The concentration of fats and fatty acids was measured by gas chromatography. The difference in concentration between tissue from Jamaicans and Chicagoans was determined by Student's 't' test.

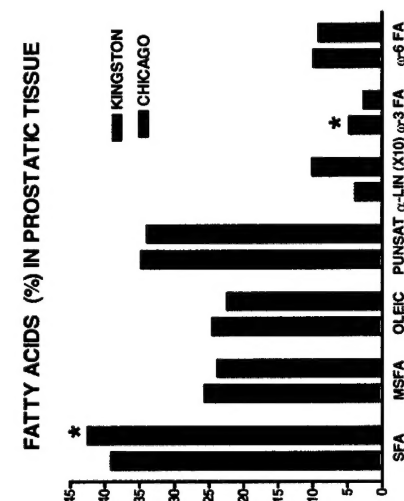
Results

In Jamaican blacks the concentration of total saturated fatty acids was lower (39.0 v 42.4% p < 0.001), that of omega-3 fatty acids and the ratio of omega-3 to omega-6 fatty acids were higher (4.7 v 2.6%, p < 0.005; 0.54 v 0.30, p < 0.02 respectively) than in prostates from American blacks. There were no differences in the concentrations of total unsaturated, mono-unsaturated and poly-unsaturated fats, oleic and α -linolenic acids or in the ratio of polyunsaturated to saturated fatty acids. (Fig 1)

Fatty acid composition of prostatic tissue from blacks in Jamaica and Chicago.

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Fatty Acid Ratios in Prostatic Tissue

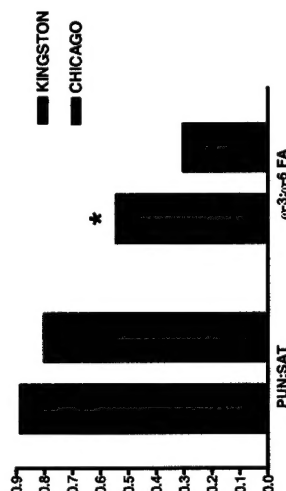


Figure 1

KEY

SFA
MSFA
OLEIC
PUNSAT
α-LIN
ω-3 FA
ω-6 FA
PUN:SAT
Ratio ω-3 to ω-6 fatty acids
p < 0.02

Discussion

Data on the association between prostate cancer and fats are inconsistent. Many studies suggest that total or saturated fat may play a role in the aetiology of prostate cancer. As a group, mono-unsaturated fats may have a positive association while there is no consistent association of poly-unsaturated fats with prostate cancer. Both positive and negative associations have been reported for individual polyunsaturated fatty acids (eg. α -linolenic and eicosapentaenoic acid respectively). The ratio of saturated to unsaturated or of ω -6 to ω -3 fatty acids has been suggested to be a more important measure of association with prostate cancer. Fats are thought to contribute to prostate cancer risk by increasing the level of endogenous androgens or by providing substrate for generating free radicals. Increased ω -3 fatty acids and a high ratio of ω -3 to ω -6 fatty acids may lower the risk by decreasing free radical formation.

The finding of increased levels of saturated fats, lower levels of ω -3 fatty acids and a higher ratio of ω -3 to ω -6 fatty acids in prostatic tissue from US than Jamaican blacks suggest that these mechanisms may contribute to the higher prostate cancer incidence in American blacks.

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Steroid 5-alpha Reductase Type II V89L Variant Frequencies and Androgen Receptor CAG Microsatellite Lengths Among Black Men With Prostate Cancer from Nigeria, Jamaica and Chicago, Illinois (USA)

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BACKGROUND

US racial comparisons of functional variants of genes involved in androgen metabolism have not provided consistent clues as to their role in the excess mortality of blacks relative to whites. Comparative studies of blacks who reside in contrasting environmental settings may shed light on the contribution of these and other inherited factors to US racial variation in prostate cancer mortality.

OBJECTIVE

We report frequencies of the V89L variant of steroid 5-alpha-reductase type II (SRD5A2) and characteristics of the androgen receptor (AR) CAG microsatellite in incident cases of prostate cancer in black men from West Africa, Jamaica and America.

MATERIALS & METHODS

Study design – Cross-sectional study.

Population – Consecutive incident prostatic adenocarcinomas diagnosed in each of three locales, Ibadan, Nigeria, Kingston, Jamaica and Chicago, Illinois over different 12-months periods between 1998 to 2001.

Data collected – Baseline clinical and demographic data were collected along with a blood sample.

Genotyping – The V89L polymorphism was genotyped by PCR-RFLP analysis using the RSA1 enzyme on purified leukocyte DNA; CAG repeat lengths were determined by PCR amplifications

Statistical analysis – Chi-square statistics and one-way analysis of variance compared distributions of categorical and continuous characteristics, respectively. A sample of Caucasian-Americans from Chicago served as an additional comparison group.

RESULTS

GENE	VARIANT	BLACKS			WHITES
		Chicago (N=42)	Kingston (N=76)	Ibadan (N=55)	
SRD5A2	V89L	5.3	6.6	54.6	56.4
	VL (%)	31.6	46.0	31.8	27.1
	LL (%)	63.1	47.4	13.6	16.5

SRD5A2:

The V89L variant was significantly more prevalent in Chicago and Jamaican blacks than in Nigerians ($p < .001$). There were no significant differences between Nigerians and Chicago whites in distribution of genotypes ($p = .8194$) even though there were significant differences as in stage at presentation ($p < .001$). However, among black cases, a decline in prevalence of variant 'L' allele across sites paralleled the decline in proportion of localized cases.

ANDROGEN RECEPTOR (AR):

While there tended to be differences among blacks across sites ($p = .02$), CAG microsatellite lengths were generally shorter among blacks compared to Chicago whites ($p = .0035$ to $< .001$).

CONCLUSION

The SRD5A2 V89L variant correlates with less androgen activation, whereas shorter CAG repeat sequence lengths facilitate androgen uptake and transcription. Expression of associated prostate cancer disease phenotypes in US blacks and whites, if any, is likely context dependent

Percent of cases with
with clinically localized
disease in each locale:

80.9%

15.6%

51.9%

88.1%

P < .001

Research supported by grants from the
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Department of Veterans Affairs (ARCD 97-317)



Steroid 5-alpha Reductase Type II V89L Variant and Risk of Advanced Prostate Cancer in Black Men from Nigeria, Jamaica and Chicago, Illinois (USA)

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Abstract 11-3710

ABSTRACT

BACKGROUND: Activity of the steroid 5-alpha reductase type II enzyme (SRD5A2) has been implicated in both risk and severity of prostate cancer. A valine to leucine substitution at codon 89 (V89L) appears to reduce enzymatic activity by approximately a third and, thus, may be protective relative to wildtype. Blacks in the US generally have the highest rates of prostate cancer in the world. However, the relative roles of heredity and the environment with respect to these rates are not well understood. Therefore, we examined the relation of the V89L variant to cancer stage among black men with prostate cancer living in Ibadan, Nigeria, Kingston, Jamaica and Chicago, IL.

MATERIALS & METHODS: Series of incident cases of prostate cancers were prospectively identified at participating hospitals in each site. Baseline clinical data along with pre-treatment blood samples were collected at time of enrollment. Using DNA extracted from peripheral leukocytes, the V89L polymorphism was genotyped by PCR-RFLP analysis using the PstAI enzyme and electrophoretic separation of DNA fragments. Chi-square analysis compared stage (localized vs. regional/distant) to genotype (VV, VL, LL) within and across sites. Logistic regression accounted for simultaneous effects of age and site, the latter incorporated into the model as dummy variables.

RESULTS: 156 Subjects were identified (38, 76 and 42 from Chicago, Kingston and Ibadan, respectively). Advanced cancers tended to be underrepresented among homo and heterozygous V89L mutants in Chicago and Jamaica (P = .0388 and .1138, respectively), but not in Ibadan (P = 0.49). **Figure 1.** After controlling for age and site, the V89L variant was significantly associated with a lower risk of advanced prostate cancer across sites (vs., wildtype, OR [95% CI] = 0.74 [0.22, 2.53] AND 0.27 [0.08, 0.96] for VL and LL, respectively, P trend = 0.01). Information regarding either PSA or tumor differentiation was not available for 15 of the 42 subjects from Ibadan. Consequently, neither variable was incorporated into the final model.

CONCLUSIONS: Functional mutations involving SRD5A2 such as the V89L variant may play a role in prostate cancer disease severity in black men. Furthermore, the role may be influenced by environmental and social settings.

Research supported by grants from the Department of Defense (DAMD1700-1-0029) and the Department of Veterans Affairs (ARCD 97-317)

Relative Odds [95% CI]¹ of Advanced (Regional/Distant) Stage Prostate Cancer

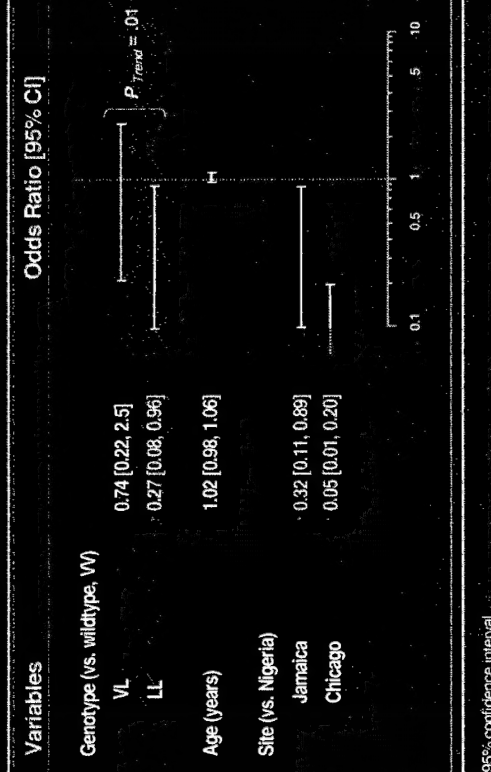


FIGURE 1. Odds ratio and 95% confidence intervals for the association between V89L genotype and risk of advanced stage prostate cancer controlling for age and site.

References

1. Merrill RM, Brawley OW. Prostate cancer incidence and mortality rates among whites and black men. *Epidemiology*. 1997;8:126-131.
2. Rebbeck TR. Inherited genotype and prostate cancer outcomes. *CEBP* 2002;11:945-952.
3. Pearce CL, et al. Steroid 5-alpha reductase type II V89L substitution is not associated with risk of prostate cancer in a multiethnic population study. *CEBP* 2002; 11:417-418.